Institute Report No. 292

Mutagenic Potential of Diethyleneglycol Dinitrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test

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GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

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The mutagenic potential of diethyleneglycol dinitrate (DEGDN) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, and TA102 were exposed to doses ranging from 5 µl/plate to 0.0016 µl/plate. The test compound was not mutagenic under conditions of this test.

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ABSTRACT

The mutagenic potential of diethyleneglycol dinitrate (DEGDN) was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, and TA102 were exposed to doses ranging from 5 µl/plate to 0.0016 µl/plate. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, Diethyleneglycol Dinitrate, DEGDN, Propellant

PREFACE

TYPE REPORT: Ames Test GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, Frederick, MD 21701-5012
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: #3E162720A835/180/TLB0

GLP STUDY NUMBER: 85014

STUDY DIRECTOR: MAJ Don W. Korte Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: SGT Steven K. Sano, BA

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, retired stability and purity data on the test compound, tissues, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Diethyleneglycol dinitrate (DEGDN)

INCLUSIVE STUDY DATES: 19 Aug - 30 Aug 85

OBJECTIVE: The objective of this study was to determine the mutagenic potential of diethyleneglycol dinitrate (LAIR Code TP047) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

CPT John W. Harbell, PhD, MSC; SGT Lillie D. Witcher, BS; SP4 John R.G. Ryabik, BS; Mr. John Dacey; and Ms. Joanne Wong provided research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP study number 85014 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr 14 July 88

DON W. KORTE JR, PhD / DATE
MAJ, MSC
Study Director

Conrad Wheeler 14 July 88

CONRAD WHEELER, PhD/ DATE
DAC
Analytical chemist

Steven Kazuo Sano 5MAR86

STEVEN K. SANO, BA / DATE
SGT, USA
Principal Investigator

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
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REPLY TO
ATTENTION OF:

SGRD-ULZ-QA (70-1n) 15 September 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 8501 4

1. This is to certify that in relation to LAIR GLP Study 8501 4, the following inspections were made:

16 August 1985	-	Protocol Review
27 August 1985	-	Plate Incorporation

2. The institute report entitled "Mutagenic Potential of Diethyleneglycol Dinitrate in the Ames Salmonella/Mammalian Microsome Mutagenicity Test,"Toxicology Series 147, was audited on 20 July 1988.

Carolyn M. Lewis

CAROLYN M. LEWIS
Chief, Quality Assurance

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Mutagenic Potential of Diethyleneglycol Dinitrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test--Sano and Korte

INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A "health effects" review conducted for the US Army Biomedical Research and Development Laboratory (USABRDL) identified numerous gaps in the toxicology database of these compounds (1). Consequently, USABRDL has tasked the Division of Toxicology, LAIR, to conduct an initial evaluation of the health effects of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity test, acute oral toxicity tests in rats and mice, acute dermal toxicity tests in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs. This report contains the results of a study that assessed the mutagenic potential of DEGDN in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

The Ames *Salmonella*/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of *Salmonella typhimurium* to detect compounds that are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating *in vivo* metabolic activation of the test compound. The Ames test is an inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (2).

This evaluation of DEGDN utilizes a revision of the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (3). Two new tester strains, a frame-shift strain (TA97) and a strain carrying an ochre mutation on a multicopy plasmid (TA102), are added to the standard tester set. TA97 replaces TA1537, TA1535 and TA1538 which are removed from the recommended set. TA98 and TA100 are retained.

Objective of the Study

The objective of this study was to determine the mutagenic potential of diethyleneglycol dinitrate (LAIR Code TP047) by using the revised Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Chemical name: Diethyleneglycol dinitrate

Code number: LAIR Code No. TP047

Physical state: Liquid

Source: Hercules Incorporated
Wilmington, Delaware

Storage: Diethyleneglycol dinitrate was received from Radford Army Ammunition Plant (Radford, VA) and assigned the LAIR Code number TP047. The test compound was stored at room temperature (21°C) until used.

Chemical Properties/Analysis: Data provided by Hercules Inc., characterizing the chemical composition and purity of the test material, are presented in Appendix A along with confirmatory analysis of the test material performed by the Division of Toxicology, LAIR (Presidio of San Francisco, CA).

Test Solvent

The positive control chemicals and the test compound were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO).

Chemical Preparation

Diethyleneglycol dinitrate was stored at room temperature (21°C) until used. On the day of dosing, 300 µl of the test compound was measured into a sterile vial and dissolved in 5.7 ml of grade I dimethyl sulfoxide to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates.

Test Strains

Salmonella strains TA97, TA98, TA100, and TA102, obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory at -80°C. Quality control tests were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (4).

Test Format

Diethyleneglycol dinitrate was evaluated for mutagenic potential according to a revised Ames method (3). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (4).

Toxicity Tests

Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of diethyleneglycol dinitrate ranging from 1.6 x 10^{-3} µl/plate to 5 µl/plate, and approximately 10^8 cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin was placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth on the plates was observed. Since none of the plates showed a decrease in the number of macrocolonies (below the number in the spontaneous reversion plates) or an observable reduction in the density of the background lawn, a maximum "limit" dose of 5 µl per plate was used in the mutagenicity test.

Mutagenicity Test

The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5, both with and without 0.5 ml of the S-9 microsome fraction. The S-9 was purchased from Microbiological Associates Inc. (Bethesda, MD). The optimal titer of this S-9, as determined by Microbiological Associates Inc., was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (5). The water used in this medium and in all reagents came from a

Technic Model 301 Reverse Osmosis Pre-Treatment Water System (Seattle, WA), LAIR SOP, OP-STX-94 (6). Plates were incubated upside down in the dark at 37°C for 72 hr. Plates were prepared in triplicate and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Maron and Ames (3). Sterility and strain verification controls were run concurrently. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The *Salmonella* strains were verified by a standard battery of tests. The integrity of the different *Salmonella* strains used in the assay was verified by the following standard tests:

-Lack of growth (inhibition) in the presence of crystal violet which indicates that the prerequisite alteration of the lipopolysaccharide layer of the cell wall is present.

-Growth in the presence of ampicillin-impregnated disks which indicates the presence of an ampicillin-resistant R Factor.

-Lack of growth (inhibition) following exposure to ultraviolet light which indicates the absence of the DNA excision-repair mechanism (for all strains except TA102).

Four known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control but may be tested with several. These compounds, benzo[a]pyrene, 2-aminofluorene, 2-aminoanthracene and 4-nitroquinoline-n-oxide, were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (7), a compound is considered mutagenic if a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count for the tester strains TA98 and TA100. A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

Maron and Ames (3) consider a compound mutagenic in tester strains TA97 and TA102 if a correlated dose response over three concentrations is achieved with the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count.

Deviations/Changes

A 72-hr rather than a 48-hr incubation period was used. According to Maron (personal communication, 1985), the additional 24-hr growth enables all of the revertant colonies, especially TA102, to be detected with the colony counter.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR archives.

RESULTS

On 23 August 1985, the toxicity of diethyleneglycol dinitrate was determined (Table 1). For this experiment all sterility, strain verification, and negative controls were normal (Table 1). No toxicity was observed after exposure of the tester strain (TA100) to the highest dose used (5 µl/plate).

Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 27-30 August 1985 (Table 2). Diethyleneglycol dinitrate did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3).

A copy of the raw data is included in Appendix B.

TABLE 1: TOXICITY DETERMINATION FOR DEGDN

GLP STUDY NUMBER 85014 23 Aug 1985 PERFORMED BY SANO/WONG

TOXICITY DETERMINATION REVERTANT PLATE COUNT (TA100)

CONCENTRATION OF TEST COMPOUND	MEAN	(1SD)	BACKGROUND LAWN*
START RUN NEGATIVE CONTROL	102	(14.0)	NL
5.0 µl/plate	72	(15.4)	NL
1.0 µl/plate	77	(5.1)	NL
0.2 µl/plate	75	(1.0)	NL
0.04 µl/plate	75	(5.5)	NL
0.008 µl/plate	80	(9.9)	NL
0.0016 µl/plate	73	(8.7)	NL
END RUN NEGATIVE CONTROL	96	(8.1)	NL

STRAIN VERIFICATION FOR TOXICITY DETERMINATION (TA100)

HISTIDINE REQUIREMENT	NG*	
AMPICILLIN RESISTANCE	G	
UV	NG	
CRYSTAL VIOLET SENSITIVITY (ZONE SIZE)	NG	(12mm)
STERILITY CONTROL	NG	

STERILITY CONTROL FOR TOXICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

* NL = Normal Lawn G = Growth NG = No Growth

TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING FOR THE MUTAGENICITY DETERMINATION OF DEGDN (TP047)

GLP STUDY NUMBER 85014 12 SEP 1985 PERFORMED BY SANO/WONG

STRAIN VERIFICATION

	OBSERVATIONS*			
STRAINS	TA97	TA98	TA 100	TA102
HISTIDINE REQUIREMENT	NG	NG	NG	NG
AMPICILLIN RESISTANCE	G	G	G	G
UV REPAIR	NG	NG	NG	G
CRYSTAL VIOLET				
SENSITIVITY	NG	NG	NG	NG
(ZONE SIZE)	(13mm)	(10mm)	(9mm)	(10mm)
STERILITY CONTROL	NG	NG	NG	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

* NL = Normal Lawn G = Growth NG = No Growth

TABLE 3: MUTAGENICITY ASSAY FOR DEGDN (TP047)

STUDY NUMBER: 85014 DATE: 12 SEPT 85 PERFORMED BY SANO/WONG

COMPOUND	DOSE	TA97	TA98	TA100	TA102
		WITHOUT S-9			
NEG CONTROL	0.0 mg/ml	57 (7.2)†	27 (4.4)	108 (12.0)	185 (6.2)
NQNO*	2.0 µg/ml			483 (335.1)	690 (53.3)
TP047	5.0 µl/plate	85 (5.1)	31 (6.2)	121 (11.9)	186 (29.1)
TP047	1.0 µl/plate	53 (12.0)	22 (2.6)	99 (2.6)	183 (7.8)
TP047	0.2 µl/plate	70 (7.5)	27 (7.2)	89 (12.0)	188 (17.3)
TP047	0.04 µl/plate	44 (4.9)	35 (1.5)	102 (3.8)	178 (7.9)
TP047	0.008 µl/plate	64 (3.2)	27 (3.5)	90 (8.2)	197 (12.5)
TP047	0.0016 µl/plate	68 (10.5)	26 (12.2)	95 (16.9)	177 (5.3)
		WITH S-9			
NEG CONTROL	0.0 mg/ml	74 (18.0)	34 (4.6)	94 (11.6)	262 (10.7)
AF*	2.0 µg/ml	352 (22.1)	1157 (168.2)	547 (14.6)	387 (27.3)
BP*	2.0 µg/ml		528 (26.7)	471 (30.5)	
AA*	2.0 µg/ml		1195 (275.1)	1132 (72.2)	
TP047	5.0 µl/plate	79 (9.1)	28 (9.2)	115 (7.6)	197 (39.6)
TP047	1.0 µl/plate	67 (12.1)	32 (5.0)	88 (6.6)	259 (24.0)
TP047	0.2 µl/plate	80 (7.5)	21 (1.0)	83 (2.5)	247 (15.6)
TP047	0.04 µl/plate	54 (6.0)	28 (5.3)	86 (15.7)	240 (13.6)
TP047	0.008 µl/plate	76 (18.7)	28 (0.0)	99 (6.8)	251 (16.6)
TP047	0.0016 µl/plate	75 (7.6)	24 (4.9)	82 (13.6)	234 (16.6)

† Values represent the mean number of revertants/plate (± standard deviation)
* NQNO = 4-nitroquinoline-n-oxide, AF = 2-aminofluorene, BP = benzo[a]pyrene, AA = 2-aminoanthracene

DISCUSSION

Certain test criteria must be satisfied before an Ames test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, alterations in the LP layer, and deficiency in DNA excision-repair (except TA102). Second, the *Salmonella* strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on formation of macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of the Ames test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, diethyleneglycol dinitrate was evaluated in the Ames test. Criteria for a positive response are a correlated dose-response relationship and a twofold increase in revertant colony counts relative to the respective negative control counts (3,4,7). Diethyleneglycol dinitrate did not induce the requisite dose-response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this test indicate that diethyleneglycol dinitrate is not mutagenic when evaluated in the Ames test.

CONCLUSION

Diethyleneglycol dinitrate was evaluated for mutagenic potential in the Ames Test, both in the presence and absence of metabolic activation, and did not induce a positive mutagenic response under conditions of this study.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846.

2. Ames BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with *Salmonella*/Mammalian Microsome Mutagenicity Test. Mutation Res 1975;31:347-364.

3. Maron DM, Ames BN. Revised methods for the *Salmonella* Mutagenicity Test. Mutation Res 1983;113:173-215.

4. Ames *Salmonella*/Mammalian Microsome Mutagenesis Test. LAIR Standard Operating Procedure OP-STX-1, Presidio of San Francisco, California: Letterman Army Institute of Research, 15 November 1983.

5. Vogel HJ, Bonner DM. Acetylornithinase of *E. coli*: Partial purification and some properties. J Biol Chem 1956;218:97-106.

6. Operation of the Technic Model 301 Reverse Osmosis Pre-Treatment Water System and the Corning Model MP-1 Glass Still. LAIR Standard Operating Procedure OP-STX-94, Presidio of San Francisco, California: Letterman Army Institute of Research, 29 July 1985.

7. Brusick D. Genetic toxicology. In: Hayes AW, ed. Principles and methods of toxicology. New York: Raven Press, 1982:223-272.

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Appendix A: CHEMICAL DATA

Chemical name: Ethanol, 2,2'-oxybisdinitrate

Alternate chemical name: Diethyleneglycol dinitrate (DEGDN)

Chemical Abstracts Service Registry No.: 693-21-0

LAIR Code No.: TP047

Chemical structure:

$$O_2N\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}NO_2$$

Molecular formula: $C_4H_8N_2O_7$

Molecular weight: 196

Physical state: Pale yellow liquid

Density (g/cm^3): 1.38[1]

Analytical data: Refer to the attached data sheet, ARRCOM Form 213R. The compound chromatographed as a single peak (retention time 5.4 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% acetonitrile; flow rate, 0.9 ml/min; detection wavelength, 205 nm.[2] NMR (300 MHz, CD_3CN): 3.75 δ (complex multiplet, 4H,-CH_2-O-CH_2-), 4.61 complex

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[1] Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland; US Army Medical Bioengineering Research and Development Laboratory, 1983; DTIC No. ADA127846, p. 17.

[2] Wheeler CR. Toxicity Testing of Propellants. Laboratory Notebook #85-12-023, p. 31. Letterman Army Institute of Research, Presidio of San Francisco, California.

Appendix A (cont.): CHEMICAL DATA

multiplet, 4H,-CH_2ONO_2).[3] Additional singlet signals of approximately equal intensity were observed at 2.08 d, and were due to sample impurities. Integration of all signals in the spectrum demonstrated that the sample contained 96.6% DEGDN. The impurities were not identified. IR(KBr): 2896, 1632, 1429, 1390, 1373,1279, 1139, 1032, 909, 857, 758, 707, 655, 572 cm^{-1}.[4]

Stability: The DEGDN was shipped containing 18% acetone (a desensitizer) and arrived at LAIR on 12 December 1984. The acetone was removed by rotary evaporation prior to studies with the propellant. Analysis of the compound one year after it was received gave the results described above.

Source: Radford Army Ammunition Plant, Radford, Virginia (prime contractor: Hercules Inc., Wilmington, Delaware).

Lot No.: RAD84M001S214

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[3] Ibid. pp. 44-48.

[4] Ibid. pp. 49-50.

Appendix A (cont.): CHEMICAL DATA

DESCRIPTION SHEET FOR EXPLOSIVES, CHEMICALS, ETC

REPORTS CONTROL SYMBOL: EXEMPT-Para 7-2a AR 335-15 | PAGE 1 OF 1

TO: | FROM: | DATE: December 5, 1984 | MATERIAL: Diethylene Glycol Dinitrate (DEGDN)

MANUFACTURER: HERCULES INCORPORATED, RADFORD ARMY AMMUNITION PLANT | CONTRACT NO.: DAAA09-77-C-4007

SECTION A - DESCRIPTION OF LOTS

FROM NUMBER	THRU NUMBER	TOTAL NO. LOTS	TOTAL NET AMOUNT ACCEPTED
RAD84M001S214	-	1	5 lbs

PLACE MANUFACTURED: RADFORD ARMY AMMUNITION PLANT, RADFORD, VIRGINIA | SPECIFICATION AND AMENDMENT/DRAWING NO.: DOD-D-64015

SECTION B - DESCRIPTION OF MATERIAL

Requirements	Limit	Results
82.2°C Potassium Iodide Starch Paper Heat Test (KI)	10 minutes minimum	12
Nitrogen, %	14.10 minimum	14.15
Water, %	Info Only	0.43
Acidity	None	None
Alkalinity	None	None

REMARKS: DEGDN is desensitized with 15% or more of acetone for a total weight of 5 lbs, and packed in a DOT 6D 5 gallon drum with a DOT 2S liner, overpacked in a DOT-6J 30 gallon capacity drum with vermiculite as a cushioning agent around the 5 gallon drum and contained in the 30 gallon drum. Requested by shipping Order AMCCOM and COR letter SMCRA dated November 28, 1984 (DOT Exemption 5704).

SECTION C - CERTIFICATION

SAMPLING CONDUCTED BY: HERCULES INCORPORATED

TESTING CONDUCTED BY: HERCULES INCORPORATED

THE ABOVE MATERIAL COMPLIES WITH ALL SPECIFICATION REQUIREMENTS AND IS CERTIFIED TRUE AND CORRECT.

12-5-84 (DATE) — (SIGNATURE) F.A.WALKER

THE ABOVE DESCRIBED LOTS ARE HEREBY ACCEPTED

Dec 6, 1984 (DATE) — (TITLE) — FOR THE COMMANDER (SIGNATURE)

ARRCOM Form 213-R, 10 Aug 77 SEQUENCE No. 374

Appendix B: INDIVIDUAL PLATE SCORES

TOXICITY DETERMINATION WITH TA100

COMPOUND	DOSE/plate	PLATE 1	PLATE 2	PLATE 3
NEGATIVE CONTROL (Start Run)		116	88	102
TP047	5.0 µl	62	65	90
TP047	1.0 µl	78	81	71
TP047	0.2 µl	74	75	76
TP047	0.04 µl	75	70	81
TP047	0.008 µl	73	75	91
TP047	0.0016 µl	71	66	83
NEGATIVE CONTROL (End Run)		101	87	101

Appendix B (cont.): INDIVIDUAL PLATE SCORES

MUTAGENICITY TESTS WITHOUT S-9

COMPOUND	DOSE/plate	TA97	TA98	TA100	TA102
NEG CONTROL (start run)		49	25	91	190
		51	31	113	183
		66	30	108	180
NEG CONTROL (END RUN)		65	21	97	176
		54	32	116	192
		54	24	123	188
NQNO*	2.0 µg			732	731
				615	630
				102	710
TP047	5.0 µl	89	29	131	153
		86	26	108	207
		79	38	125	199
TP047	1.0 µl	54	25	98	178
		65	21	102	192
		41	20	97	179
TP047	0.2 µl	78	19	90	184
		70	31	101	173
		63	32	77	207
TP047	0.04 µl	50	36	104	172
		42	35	98	175
		41	33	105	187
TP047	0.008 µl	62	27	92	210
		63	23	81	196
		68	30	97	185
TP047	0.0016 µl	78	37	107	173
		57	29	76	183
		69	13	103	175

* 4-nitroquinoline-n-oxide

Appendix B (cont.): INDIVIDUAL PLATE SCORES

MUTAGENICITY TESTS WITH S-9					
COMPOUND	DOSE/plate	TA97	TA98	TA100	TA102
NEG CONTROL		65	34	89	262
(Start Run)		45	38	83	271
		70	33	90	252
NEG CONTROL		97	26	86	273
(End Run)		85	38	108	266
		80	37	110	246
2-aminofluorene	2.0 µg	327	1347	530	358
		362	1095	557	392
		368	1028	553	412
benzo(a)pyrene	2.0 µg		500	492	
			532	485	
			553	436	
2-aminoanthracene	2.0 µg		1186	1198	
			924	1144	
			1474	1055	
TP047	5.0 µl	86	18	120	238
		69	30	118	193
		83	36	106	159
TP047	1.0 µl	80	32	89	260
		56	37	81	282
		66	27	94	234
TP047	0.2 µl	72	20	81	245
		87	21	86	264
		81	22	83	233
TP047	0.04 µl	60	32	91	254
		48	30	98	238
		53	22	68	227
TP047	0.008 µl	70	28	107	233
		61	28	94	253
		97	lost	97	266
TP047	0.0016 µl	84	30	80	236
		70	21	69	217
		72	22	96	250

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